Formulation and comparative evaluation of microspheres of *Centella asiatica* L. and *Syzygium aromaticum* for their antibacterial and neuroprotective activity.

A Synopsis

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INTRODUCTION

Plants occupy a unique position in this planet since they are the foundation to life on the earth. They are the primary producers in all food chains. Plants directly supply 90% of human calorific intake and 80% of proteins intake. Plants are being used as a potential source of medicine for time immemorial. India is the source of wealth of a large number of medicinal herbs and they are opting for the traditional medicine methods. Hence, it is imperative that this great natural resource is augmented and utilized in accordance with the development of technology and the needs of human beings. The current millennium sets the goal of side effect free treatment by way of neutraceuticals. The global interest on traditional medicine has gained importance as a number of plants have been cited in curing diseases successfully. Medicinal plants have been a valuable source of natural active phyto-consituents that play an important role in treatment of many human diseases. This is mainly because these herbs/plants are relatively economical, easily available and their uses were decided on ancestral experience. In addition, synthetic drugs are not only expensive and inadequate for the treatment of disease, but are also faced with adulteration and side effects. Therefore, medicinal plants have been useful in the development of new drugs and continue to play valuable role in the drug discovery process. Medicinal plants are an integral part of the ethno botanical aspects of the people of Asia for centuries. The modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening. Advanced microbial and chemical methods can synthesize medicinal and aromatic compounds, but the cost in many cases is expensive. However, synthetic medicine can cause side effects and as a result, people are more favorable to use natural compounds obtained from plants.

Introduction to antiulcer activity:

Gastric ulcer is one of the common diseases in human population. Due to rapid development and civilization constraints including a stressed lifestyle, the incidences of ulcer are increasing. In view of the progress of western medicine not only new synthetic drugs but also herbal drugs have to fulfil the international requirements on quality, safety and efficacy. Helicobacter pylori infection is associated with an increased risk of developing upper gastrointestinal tract diseases. However, treatment failure is a major cause of concern mainly due to possible recurrence of infection, the side

effects, and resistance to antibiotics. In the present study, antiulcer activity were evaluated of two selected plants *Centella asiatica* (L.) and Syzygium aromaticum used traditionally for ulcer healing.

Introduction to Neuroprotective activity:

Neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, ischemia and traumatic injury are characterized by progressive neuronal loss and dysfunction. Many neuroprotective agents modulating cellular responses against noxious stimuli, such as oxidative stresses, thereby having anti-inflammatory and antiapoptotic activity have been studied to develop the therapeutics for neurodegenerative diseases. Recently, the phenolic compounds widely spread in medicinal plants have drawn attention as potential neuroprotective agents. . In the present study, antiulcer activity were evaluated of two selected plants Centella asiatica (L.) and Syzygium aromaticum used traditionally for neurological disorders.

Microsphere formulation:

Administration of medication via micro particulate systems is advantageous because microspheres can be ingested or injected and; they can be tailored for desired release profiles and used site-specific delivery of drugs and in some cases can even provide organ-targeted release. Considerable research efforts are spent in the development of non-steroidal anti-inflammatory drugs (NSAIDs) and the conventional treatment available may produce gastrointestinal side effects along with other associated adverse effects. Multi-unit micro particulate dosage forms may solve those problems because they spread more uniformly in the gastrointestinal tract which facilitates uniform reliable drug release in a sustained manner. Therefore, multi-unit micro particulate dosage forms (microspheres) nowadays is most recommended by the healthcare professionals when drug safety and patient compliance is a major concern Therefore, dosage form of microspheres has been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release .

Brief Description of selected plants

Centella asiatica (L)

Centella asiatica (Family- Apiaceae) is a psychoactive medicinal plant being used from centuries in Ayurvedic system of medicine as a Medhya Rasayana. Many traditional medicines in use are derived

from medicinal plants, minerals and organic matter. Centella asiatica L. has been used as a medicinal herb for thousands of years in India, China, Sri Lanka, Nepal and Madagascar. Centella asiatica is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells. Centella asiatica has a long history in ancient Ayurvedic remedy, used in wound healing, cleansing for skin problem and digestive disorders and effective in treatment of stomach ulcers, mental fatigue, diarrhea, epilepsy, hepatitis, syphilis and asthma . C. asiatica is also rich in flavanoids and terpenoids compounds among them asiatic acid, asiaticoside, madecassoside is well characterized for its pharmacological value.

Syzygium aromaticum

Clove is the dried reddish brown flower bud of Syzygium aromaticum (Family: Myrtaceae). It contains volatile oil (14% - 21%), tannin (10% - 13%), phenol, sesquoterpene ester and alcohol. The most important constituent of clove is the phenylpropene eugenol which gives this spice its pungent, distinctive aroma. Eugenol makes up 70 % to 90 % of the essential oil and 15 % of the dry weight of clove buds Studies have attributed therapeutic properties to clove such as aphrodisiac, stomachic, carminative, antispasmodic, anti-inflammatory, antioxidant, anti-hyperglycemic, anti-stress, antimutagenic, and allelopathic, as well as antiseptic and anesthetic to relieve toothache among other pains. It is also reported to be useful in conceiving in high doses and acts as a contraceptive in low doses and useful in cataract. But there are few studies about the effect of clove on the neuronal activities.

REVIEW OF LITERATURE

1. Hong-Mei Zheng__et al., (2016) investigated In vitro and In vivo Anti-Helicobacter pylori Activities of Centella asiatica Leaf Extract. The aim of this study was to investigate the activities of Centella asiatica leaf extract (CAE) against H. pylori both in vitro and in vivo. The minimum inhibitory concentrations (MICs) against 55 clinically isolated strains of H. pylori were tested using an agar dilution method. The MICs of CAE ranged from 0.125 mg/mL to 8 mg/mL, effectiveness in inhibiting H. pylori growth was 2 mg/mL. The anti-H. pylori effects of CAE in vivo were also examined in H. pylori-infected C57BL/6 mice. CAE was orally administrated once daily for 3 weeks at doses of 50 mg/kg and 250 mg/kg. CAE at the 50 mg/kg dose significantly reduced H. pylori colonization in mice gastric mucosa.

2. Ved Prakash1 **et al., (2017)** studied *Centella asiatica* is traditional Ayurveda medicine widely used in India and across Asia for treating a variety of diseases. The aerial parts and roots are used for medicinal purpose, and its chemical constituents have wide therapeutic applications in areas of antimicrobial, anti-inflammatory, anticancer, neuroprotective, antioxidant, and wound healing activities. Many of its uses have been proven scientifically, and bioactive ingredients have been validated.

3. Saowanee Wannasarita,b et al., (2017) investigated Raft-forming gastro-retentive formulations based on Centella asiatica extract solid dispersions for gastric ulcer treatment. Liquid raft-forming formulations comprising solid dispersions of glycoside-rich Centella asiatica extract and Eudragit® EPO (GR-SD) were developed to achieve prolonged delivery of the glycosides, asiaticoside (AS) and madecassoside (MS) in the stomach and thus increase the effectiveness of gastric ulcer treatment. Solid dispersions of GR extract and Eudragit® EPO (GR-SD, weight ratio 1:0.5) resulted in the highest solubility of AS (41.7 mg/mL) and MS (29.3 mg/mL) and completed dissolution of both glycosides occurred in SGF within 10 min. The optimized raft-forming formulation was composed of alginate (2%), HPMC K-100 (0.5%), GR-SD (1.2%), and calcium carbonate (0.5%) as a calcium source and carbon dioxide producer. The formulation provided sufficient raft strength (> 7.0 g), rapid floating behavior in SGF (~30 s), and sustained release of AS (more than 80%) and MS (85%) over 8 h. GR-SD-based formulations administered once

daily to rats for two days at a dose of 10 mg AS/kg reduced the severity of gastric ulcer induced by indomethacin with a greater curative efficacy than those of unformulated GR extract and a standard antiulcer agent: lansoprazole ($p < 0.05$). These findings demonstrate that GR-SD-based raft-forming systems offer significant promise for improving the treatment of gastric ulcers induced by non-steroidal anti-inflammatory drugs.

4. M. A. Abdulla et al., (2010) studied that to evaluate the anti-ulcerogenic activity of ethanol extract of *Centella asiatica* against ethanol-induced gastric mucosal injury in rats. Five groups of adult *Sprague Dawley* rats were orally pre-treated respectively with carboxymethyl cellulose (CMC) solution (ulcer control group), Omeprazole 20 mg/kg (reference group), and 100, 200 and 400 mg/kg *C. asiatica* leaf extract in CMC solution (experimental groups), one hour before oral administration of absolute ethanol to generate gastric mucosal injury. Rats were sacrificed and the ulcer areas of the gastric walls were determined. Grossly, the ulcer control group exhibited severe mucosal injury, whereas pre-treatment with *C. asiatica* leaf extract exhibited significant protection of gastric mucosal injury. Histological studies revealed that ulcer control group exhibited severe damage of gastric mucosa, along with edema and leucocytes infiltration of submucosal layer compared to rats pre-treated with *C. asiatica* leaf extract which showed gastric mucosal protection, reduction or absence of edema and leucocytes infiltration of submucosal layer.

5. Sunanda BPV et al., (2014) suggests that long term administration of CA prevents scopolamine-induced cognitive impairment and associated oxidative stress. Thus, the use of CA is promising for the treatment of Alzheimer's diseases (AD) and other neurodegenerative disorders.

6. Coralie Pavesi et al., (2018) studied that Clove contains bioactive compounds which can be potentially used for medicinal purposes. Eugenol is the major component of the clove essential oil and it is the main responsible compound for the antimicrobial activity of that extract. This study showed no sufficient evidence to support the synergistic effect of other compounds in the clove oil as both the extract and the eugenol standard showed similar pattern of antimicrobial activity. Further studies are required, before the incorporation of eugenol into a drug formulation. The exact mechanism of action must be fully understood and a pharmacological evaluation is needed to determine the effective dosage and minimise any potential side effects.

7. Rami B. Kassab et al., (2014) wSorked on is to study the possible neuroprotective effect of aqueous extract of clove (*Syzygium aromaticum*) on ions. The results presented here, indicate that the toxic effects of AlCl3 could be mediated through modifying the intracellular brain ions homeostasis, cholinergic dysfunction and oxidative damage in rat brains which may lead to impaired neuronal function. Taken together the results of this study also showed that clove offers neuroprotection against AlCl3-induced neurotoxicity.homeostasis, acetylcholinesterase (AChE) activity and the oxidative status in different brain regions of adult male Wistar albino rats which were intoxicated by aluminium chloride (AlCl3).

8. Chang peng et al.,(2022) analyzed the chemical compositions of *S. aromaticum* extracts, and then confirmed their activities on both antibiotic-sensitive and -resistant *H. pylori* strains. In addition, the mechanisms of action of ***S. aromaticum*** extracts against ***H. pylori*** were found to be destroying the bacterial structure, down-regulating the expression of virulence genes, and interfering TAC and pyruvate metabolic pathways.

9. A Tomas et al., (2022) carried out his study on ***S. aromaticum*** extracts which contain a diverse array of secondary metabolites, like flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenyl propenes, eugenol, eugenol acetate, and gallic acid, with a wide range of beneficial health-promoting activities. These metabolites have active biological properties and unique aromatic structures and are safe for use in food and medicinal applications. Clove metabolites are considered as new beneficial natural antimicrobial agents to potentially combat cytotoxin genes-producing drug-resistant ***Helicobacter pylori***, gastrointestinal disorders, anti-inflammatory, antithrombotic, antidiabetic, anesthetic, and possess insect-repellent properties.

10. Chatterjee et al., (2001), Protein loaded polystyrene and albumin microspheres loaded with RBC were prepared by chemical crosslinking method. In vitro drug release profiles of polymer-drug were found.

11. Misbah Hameed et al., (2021) showes that Clove oil-encapsulated NFs for the topical treatment of wounds were successfully prepared using CS and PEO as polymers. Prepared NFs were characterized using SEM, FTIR, and XRD techniques.

12 Da Hyun Jung et al., (2020) worked on Effect of β-caryophyllene from Cloves Extract on *Helicobacter pylori* Eradication in Mouse Model

13. Mohamed Aboubakr et al., (2018) shows that Neuroprotective Effects of Clove Oil in Acrylamide Induced Neurotoxicity in Rats. Clove oil (CO) is known for its antioxidant properties and has great potential for pharmaceutical, food, cosmetic and agricultural uses. Acrylamide (AA) as a potent neurotoxic agent and human exposure to AA involves dietary intake of carbohydrates processed at high temperature. This study evaluated if CO administration could protect against neurotoxicity induced by AA in rats.

14. Hashim, P (2011) worked on Centella asiatica in food and beverage applications and its potential antioxidant and neuroprotective effect.

15. Ilkay Erdogan Orhan (2012) studied on *C. asiatica* (gotu kola) has been reported to have a comprehensive neuroprotection by different modes of action such as enzyme inhibition, prevention of amyloid plaque formation in Alzheimer's disease, dopamine neurotoxicity in Parkinson's disease, and decreasing oxidative stress. Therefore, *C. asiatica* could be suggested to be a desired phytopharmaceutical with neuroprotective effect emerged froms traditional medicine.

16. Chuen Lung Cheng et al., (2004) worked on the healing effects of Centella asiatica water extract (CE) and asiaticoside (AC), an active constituent of CE, on acetic acid induced gastric ulcers (kissing ulcers) in rats were examined.

17. Maneesh Jaiswal et al., (2016) worked on Nanoemulsions loaded Carbopol® 934 based gel for intranasal delivery of neuroprotective *Centella asiatica* extract: in–vitro and *ex–vivo* permeation study.

The objective of the present study was to develop a formulation for sustained release of phytotherapeutics through nasal membrane for brain targeting (anti-Alzheimer) in order to overcome their low residence time and poor nasal permeability issues. A dual approach containing nanoemulsions within gel (NE-gels) was used to achieve the objectives utilizing biocompatible biomaterials. Nanoemulsions were characterized for particle size and zeta potential. NE-gels loaded with Centella asiatica (CA) extract were developed using Carbopol® 934 and were

characterized for rheological properties (spreadability, viscosity), drug release and prolong anti-oxidant profile.

OBJECTIVE OF REASERCH

The main objective to formulate microsphere as formulation of centella asiatica and syzygium aromaticum and perform comparative evaluation for their antibacterial and neuroprotective activity.Specific objectives include:

- Selection and authentication of plants.
- To study Pharmacognostical characters of *Centella asiatica* and *Syzygium aromaticum.*
- Development and Evaluation microspheres.
- To carryout pharmacological evaluation

HYPOTHESIS

- By taking idea from literature review we are decided to work on the preparation of herbal formulation.
- Due to long term treatment of ulcer and neurological disorders, the herbal medicinal therapy can be the best alternative over synthetic drugs to minimize the occurrence of side effects.
- In the traditional treatment many plants are used for antibacterial and neuroprotective activity but some of them are away from pharmaceutical segment due to their formulation problems.
- The purpose of the study is to examine such potent medicinal plant/plants and creating pharmaceutical formulations by overcoming the all problems which will be helpful in the management of various ulcer and neurological disorders disorders.

RESEARCH METHODOLOGY

The methodology of research work will be selected on the basis of literature survey as follows:

- Collection, Identification and Authentication of plant material
- Pharmacognostic evaluation of plant material
- Processing of plant material
- Extraction of plant material
- Phytochemical screening of plant material
- Phytochemical qualitative analysis of selected plant extract will be carried out by preliminary phytochemical test i.e presence of Carbohydrates, Tannin, Alkaloids, Glycosides, Flavonoides, Steroids, Amino acid and proteins etc.
- Phytochemical quantitative analysis of extract: The total phenolic & flavonoids content will be determined by phytochemical quantitative analysis.
- Development of microspheres
- Evaluation of microsphere.
- Pharmacological screening of microspheres for antibacterial and neuroprotective activity by animal model as per reference standard methods.
- Interpretation of data and its presentation

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